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3. (Amended) The method of claim 1, wherein said target RNA comprises a ribosome.

4. (Amended) The method of claim 1, wherein said target RNA is a ribosome.

Pending claims

Claims 1 to 16 are pending. Claims 14 and 15 are canceled without prejudice as non-elected, claims 2 to 4 are amended. Upon entry of this Amendment and Response, claims 1 to 13 and 16 are presented for examination. No new matter is added by this amendment.

The present invention provides "a method for determining whether a test compound binds to a target RNA, the method comprising the steps of: (a) contacting the test compound with the target RNA and a RNA-modifying enzyme; and (b) detecting the modification of the target RNA by the enzyme and comparing the amount of modification detected to that of a standard, wherein the comparing determines whether the test compound binds to the target RNA."

Formal Matters

The Office Action objects to the disclosure because the X and Y axes are not labeled in Figure 22. Applicants submit herewith a proposed amended Figure 22 wherein the X axis is labeled with "50 μM of compound (cpd)", and the Y axis with "% inhibition of methyltransferase." Support for this amendment can be found in the specification on page 16, lines 20-24, which states "A series of compounds A-J were assayed at 50μM, compounds C-G and J showed significant (greater than 40%) inhibition of the methyltransferase. The ErmE assay can therefore be implemented in a high throughput screening format."

Rejection of Claims 2 and 4 Under 35 U.S.C. § 112 Second Paragraph

Claims 2 and 4 were rejected under 35 U.S.C. § 112, Second Paragraph for failing to particularly point out and distinctly claim the invention. The Office Action states that the "term 'sub-regions thereof'' is confusing for whether the ribosome fragment or sub-region of ribosome comprises other than the recited regions or do in fact comprise other regions of fragments or sub-regions derived from the ribosome."

Claim 2: "sub-region thereof"

For clarification, the Applicants have amended the claim as to delete the phrase "or a fragment or sub-region thereof", but intend the term "rRNA" to encompass a fragment or sub-region of a rRNA that is useful as a target RNA according to the invention. Support for this amendment is found in the specification on page 5, lines 5-16.

Claim 3. Deletion of the term "whole"

For clarification, Applicants have deleted the word "whole", but intend the term "ribosome" to encompass a whole ribosome, as where sub-regions and fragments of a ribosome are useful, according to the invention.

Claim 4: "sub-region thereof"

For clarification, the Applicants have amended the claim as to delete the phrase "fragment or sub-region thereof", but intend the term "target RNA" to encompass a fragment or sub-region of a ribosome that is useful according to the invention. Support for the amendment can be found in the Specification on page 6, lines 1-2.

Accordingly, Applicants submit that in view of the above amendments, the rejection is now moot and therefore respectfully request that the rejection be reconsidered and withdrawn.

Rejection of Claims 1 to 13 Under 35 U.S.C. § 103(a)

Claims 1 to 13 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Karn et al. (U.S. patent 6,316,194) in view of Villsen et al. (J. Mol. Biol., 286: 365-374, 1999).

The Office Action states that Karn et al. teach a method for determining whether a test compound binds to target RNA. The Office Action acknowledges that Karn et al. does not teach the use of RNA-modifying enzymes taught by the Applicants; however, it asserts that it would be obvious to combine the teachings of Villsen et al., which "teaches the mechanism of action of antibiotic, erythromycin...", with those of Karn et al. to arrive at the instant invention. Applicants respectfully traverse the rejection.

Applicants submit that neither Karn et al. nor Villsen et al. alone or in combination teach or suggest the claimed invention.

Karn et al. does not expressly or impliedly suggest the claimed invention.

It is stated in the Office Action that Karn et al. teach a method for determining if a test compound binds to target RNA comprising incubating a test compound with target RNA and an antimicrobial molecule, measuring or detecting the change or modification of said target RNA, and comparing the amount of change to that of a standard to identifying test compounds that bind to the target RNA. The Office Action states that Karn et al. does not teach RNA-modifying enzymes, "which are involved in the underlying mechanism of action of these antibiotics." Thus, it is agreed that Karn et al. does not teach or suggest RNA-modifying enzymes. While Applicants agree that the Karn patent fails to teach RNA modifying enzymes, Applicants wish to counter the statement in the Office Action that RNA modifying enzymes "are involved in the underlying mechanism of action of these antibiotics." If anything, the RNA and the enzymes are involved in conferring resistance to the antibiotics, not in the mechanism of action of the antibiotics. Applicants submit, that Karn et al. does not teach or suggest RNA-modifying enzymes and thus, does not expressly or impliedly suggest this element of the claimed invention.

Villsen et al. does not expressly or impliedly suggest the claimed invention.

It is stated in the Office Action that Villsen et al. "teaches the mechanism of action of antibiotic, erythromycin, wherein Villsen et al. teach that erythromycin acts via RNA-modifying enzyme, adenine-specific N-methyltransferase and alters or modifies ribosomal RNA (rRNA)

target site and modification of rRNA by erythromycin methyl transferase confers resistance." Applicants submit that Villsen et al. do not teach the mechanism of action of antibiotics and do not teach that erythromycin acts via an RNA-modifying enzyme. Rather the reference teaches the mechanism of action of ErmE methylase in antibiotic resistance. Villsen et al. teach that RNA modifying enzymes (Erm methyltransferases) bind to 23S ribosomal RNA (rRNA) and methylate the rRNA. The reference further teaches that methylation of 23S rRNA by Erm methyltransferases, confers resistance to macrolide antibiotics (i.e. erythromycin). Villsen et al. looked at the effects of individual nucleotide substitutions in 23S rRNA to identify nucleotides that define the ErmE methyltransferase recognition motif. They mutated several residues surrounding adenosine 2058 of 23S rRNA, which is methylated by ErmE methyltransferase, and were able to identify a nucleotide motif recognized by ErmE methyltransferase.

Applicants submit that Villsen et al. does not teach or suggest that erythromycin changes the methylating activity of RNA-modifying enzyme ErmE methyltransferase. The reference therefore does not teach or suggest that erythromycin would change the amount of modification of the rRNA target of ErmE enzyme.

Villsen et al. does not teach that erythromycin inhibits binding of RNA modifying enzymes to the 235 rRNA, or that erythromycin inhibits methylation of 23S RNA. It is stated in the introduction of the Villsen et al. reference starting on page 365, paragraph 2 that:

> "A2058 and nucleotides that are nearby in the primary and higher-order rRNA are involved in the binding of erythromycin and other MLS antibiotics. Mutations at these nucleotides confer antibiotic resistance, presumably by reducing the strength of the drug-rRNA interaction. Modification of the rRNA by Erm methyltransferase probably confers resistance by the same mechanism."

Thus, there is no teaching of a molecule (the RNA modifying enzyme) that binds to target RNA and can change the amount of modification of the target RNAs, the RNA modifying enzyme, as required by the instant claims. Villsen et al. simply suggests that modification of A2058 by methylation, or mutation of residues surrounding A2058 confer resistance to antibiotics by

reducing the strength of the drug-rRNA interaction. Given that there is no teaching or suggestion in the reference that erythromycin can change the modification of rRNA by ErmE methyltransferase, the combination of this reference with the teachings of Karn et al. will not provide the claimed invention, which relies on differences in the amount of modification of target RNA by the test compound. Because Villsen et al. fails to provide the teaching missing from the Karn et al. reference, the combination of these two references can not render the claimed invention obvious.

The Office Action does not provide a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references.

Applicants submit that the Office Action does not provide a motivation to combine Villsen et al. with the reference of Karn et al. The Office Action asserts that it would have been obvious to combine the method of detecting a test compound as taught by Karn et al. with the RNA-modifying enzymes, as taught by Villsen et al.,

> "because Karn et al states that ' In most biological systems, the functions of RNA is often determined by the interactions between highly conserved RNA structures. In many instances it is desirable to develop drugs that bind RNA at sites of conserved structure to act as competitive inhibitors of the RNA function that is derived from various RNA interactions. These types of drugs have potential applications in a wide range of diseases including bacterial, viral, and fungal infections. Many antibiotics function by inhibiting protein synthesis, and it has become increasingly clear that many do so by acting at the level of ribosomal RNA'."

The Office Action States that "one such alternative mechanism of action of antibiotics expressly motivated by Villsen et al. is to provide 'a better understanding of the three-dimensional structure of this RNA motif [which] will facilitate the design of small molecules of homologous shape that can be used to bind and inhibit the active site of the ErmE methyltransferase enzyme. This could lead to an effective means of combating MLS resistance'."

Applicants submit that this statement by Villsen et al. refers to design of molecules that mimic the ErmE methyltransferase recognition motif of ribosomal RNA. Specifically, the

reference states that the suggested approach "will facilitate the design of small molecules of homologous shape that can be used to bind and inhibit the active site of the ErmE methyltransferase enzyme." Villsen et al. does not to or suggest the design of molecules that bind to rRNA and alter the amount of modification (e.g., methylation) of the rRNA by the RNA modifying enzyme, as required by the claims. There is no teaching or suggestion in Villsen et al. that antibiotics change the modification of rRNA by ErmE methyltransferase. Thus, the teachings of Villsen et al. do not provide a motivation to combine RNA-modifying enzymes with the method of Karn et al. In addition, Karn et al. only makes a general statement that it is "desirable to develop drugs that bind RNA at sites of conserved structure to act as competitive inhibitors of the RNA function that is derived from various RNA interactions." There is no suggestion in the reference that an antibiotic could alter the amount of target RNA modification by an RNA-modifying enzyme or that this could form the basis of an assay.

In view of the above, Applicants respectfully submit that there is no motivation to combine the cited references. The mere fact that references can be combined does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *Berghauser v. Dann, Comr. Pats.*, 204 U.S.P.Q. 393 (Dist. DC 1979); *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 221 U.S.P.Q. 929 (Fed. Cir. 1984). Citing references which merely indicate that isolated elements and/or features recited in the claims are known is not a sufficient basis for concluding that the combination of claimed elements would have been obvious. *Ex parte Hiyamizu*, 10 U.S.P.Q.2d 1393 (Bd. Pat. App. & Inter. 1988). Further, as discussed above, even if the references are combined, they do not result in the invention of claim 1 or of claims 2-13 that depend on it. Villsen et al. gives ErmE binding, if anything. Accordingly, Applicants respectfully submit that the rejection is improper and request that the rejection be reconsidered and withdrawn.

Rejection of Claims 1 to 11 and 16 Under 35 U.S.C. § 103(a)

Claims 1 to 11 and 16 were rejected under 35 U.S.C. § 103(a) as being obvious over Stern et al. (USPN. 5,3712,096) and in view of Villsen et al. (J. Mol. Biol., 286: 365-374, 1999). The Office Action states that Stern et al. teach a method for determining a test compound binds to target RNA. The Office Action acknowledges that Stern et al. does not teach the use of RNA-modifying enzymes taught by the Applicants; however, the Office Action asserts that it would be obvious to combine the teachings of Villsen et al., which "teaches the mechanism of action of antibiotic, erythromycin...", with those of Stern et al. to arrive at the instant invention.

Applicants respectfully traverse the rejection.

Applicants submit that neither Stern et al. nor Villsen et al. alone or in combination, expressly or impliedly provide the claimed invention.

Stern et al. does not expressly or impliedly suggest the claimed invention.

It is stated in the Office Action that Stern et al. "teach a method for determining a test compound that binds to target RNA comprising incubating a test compound with target RNA or an analog molecule, and a ligand molecule, measuring or detecting the disruption or modification of said target RNA binding complex, and comparing the amount of change binding complex before and after the addition of the test compound to the target RNA. The Office Action recognizes that Stern et al. does not teach RNA-modifying enzymes, "which are involved in the underlying mechanism of action of these antibiotics." It is agreed that Stern et al. does not teach or suggest RNA-modifying enzymes. However, Applicants reiterate a counter to the statement in the Office Action that RNA modifying enzymes "are involved in the underlying mechanism of action of these antibiotics." Applicants submit that Stern et al. does not teach or suggest RNA-modifying enzymes and thus, does not teach or suggest that a ligand used in their assay could be an RNA-modifying enzyme.

Villsen et al. does not expressly or impliedly suggest the claimed invention.

It is stated in the Office Action that Villsen et al. "teaches the mechanism of action of

antibiotic, erythromycin, wherein Villsen et al. teach that erythromycin acts via RNA-modifying enzyme, adenine-specific N-methyltransferase and alters or modifys ribosomal RNA (rRNA) target site and modification of rRNA by erythomycin methyl transferase confers resistance." As discussed above, in relation to Villsen et al. and Karn et al., Applicants submit that Villsen et al. does not teach the mechanism of action of antibiotics and does not teach that erythromycin acts via an RNA-modifying enzyme. In contrast the reference teaches the mechanism of action of ErmE methylase. Villsen et al. teach that RNA modifying enzymes (Erm methyltransferases) bind to 23S ribosomal RNA (rRNA) and methylate the rRNA. The reference further teaches that methylation of 23S rRNA by Erm methyltransferases, is what confers resistance to macrolide antibiotics (i.e. erythromycin). Villsen et al. looked at the effects of individual nucleotide substitutions in 23S rRNA to identify nucleotides that define the ErmE methyltransferase recognition motif. They mutated several residues surrounding adenosine 2058 of 23S rRNA, which is methylated by ErmE methyltransferase, and were able to identify a nucleotide motif recognized by ErmE methyltransferase.

Villsen et al. does not teach that erythromycin inhibits binding of RNA modifying enzymes to the 23S rRNA, or that erythromycin inhibits methylation of 23S RNA. It is stated in the introduction of the Villsen et al. reference starting on page 365, paragraph 2 that:

> "A2058 and nucleotides that are nearby in the primary and higher-order rRNA are involved in the binding of erythromycin and other MLS antibiotics. Mutations at these nucleotides confer antibiotic resistance, presumably by reducing the strength of the drug-rRNA interaction. Modification of the rRNA by Erm methyltransferase probably confers resistance by the same mechanism."

Thus, there is no teaching of a molecule that binds to target RNA (e.g,. rRNA) and can change the amount of modification of the target RNAs, the RNA modifying enzyme, as required by the instant claims. Villsen et al. simply suggests that modification of A2058 by methylation, or mutation of residues surrounding A2058 confer resistance to antibiotics by reducing the strength of the drug-rRNA interaction. Given that there is no teaching or suggestion in the reference that erythromycin can or would alter the modification of rRNA by ErmE methyltransferase,

combination of this reference with the teachings of Stern et al. does not provide the claimed invention, which relies on differences in the amount of modification of target RNA by the test compound. Because Villsen et al. fails to provide the teaching missing from the Stern et al. reference, the combination of these two references can not render the claimed invention obvious.

The Office Action does not provide a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references.

Applicants submit that the Office Action does not provide a motivation to combine Stern et al. with the reference of Villsen et al. The Office Action asserts that it would have been obvious to combine the method of detecting a test compound as taught by Stern et al. with the RNA-modifying enzymes, as taught by Villsen et al.,

> "because Stern et al. states that 'most antibiotics that inhibit protein synthesis act directly on ribosomes. Several factors, all related to the structural complexity of ribosome, complicate screening assays that rely on binding of a potential drug candidate to a ribosomal target. Despite the complex structures and numerous associated proteins of complete ribosomes, we have discovered small oligoribonucleotide analogs that mimic small domains of parental RNAs and that can fold and function autonomously for purposes of screening assays. One such potent antibiotic analog mechanism of action, expressively motivated by Villsen et al. is to provide 'a better understanding of the three-dimensional structure of this RNA motif [which] will facilitate the design of small molecules of homologous shape that can be used to bind and inhibit the active site of the ErmE methyltransferase enzyme. This could lead to an effective means of combating MLS resistance'."

Applicants submit that the statement by Villsen et al. refers to design of molecules that mimic the ErmE methyltransferase recognition motif of ribosomal RNA. Specifically, the reference states that the suggested approach "will facilitate the design of small molecules of homologous shape that can be used to bind and inhibit the active site of the ErmE methyltransferase enzyme." Villsen et al. does not teach or suggest the design of molecules that bind to rRNA and alter the amount of modification (e.g., methylation) of the rRNA by the RNA modifying enzyme as required by the claims. There is no teaching or suggestion in Villsen et al.

that antibiotics change the modification of rRNA by ErmE methyltransferase. Thus, the teachings of Villsen et al. do not provide a motivation to combine RNA-modifying enzymes with the method of Stern et al. In addition, Stern et al. only makes a general statement "most antibiotics that inhibit protein synthesis act directly on ribosomes." There is no suggestion in the reference that antibiotic could alter the amount of target RNA modification by an RNA-modifying enzyme or that this could form the basis of an assay.

In view of the above, Applicants respectfully submit that there is no motivation to combine the cited references. The mere fact that references can be combined does not render the resultant combination obvious unless the prior art also suggest the desirability of the combination. *Berghauser v. Dann, Comr. Pats.*, 204 U.S.P.Q. 393 (Dist. DC 1979); *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 221 U.S.P.Q. 929 (Fed. Cir. 1984). Citing references which merely indicate that isolated elements and/or features recited in the claims are known is not a sufficient basis for concluding that the combination of claimed elements would have been obvious. *Ex parte Hiyamizu*, 10 U.S.P.Q.2d 1393 (Bd. Pat. App. & Inter. 1988). Further, as discussed above, even if the references are combined, they do not result in the invention of claim 1 or of claims 2-13 that depend on it. Villsen et al. gives ErmE binding, if anything. Accordingly, Applicants respectfully submit that the rejection is improper and request that the rejection be reconsidered and withdrawn.

CONCLUSION

All rejections raised by the Office Action have been addressed herein. Applicants therefore respectively request reconsideration of the claims. If the Examiner believes that a telephone conversation with Applicants' attorney would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record.

Respectfully submitted,

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Marked-Up Version of Claims/Spec/etc

2. (Amended) The method of claim 1, wherein said target RNA comprises a rRNA [or a fragment or sub-region thereof].

3. (Amended) The method of claim 1, wherein said target RNA comprises a [whole] ribosome.

4. (Amended) The method of claim 1, wherein said target RNA is a ribosome [or a fragment or sub-region thereof].